Selected miRNAs and mRNAs as biomarkers to Predict Response of Advanced Colorectal Cancer (CRC) to Chemotherapy

JUNTA DE ANDALUCIA
CONSEJERÍA DE IGUALDAD, SALUD Y POLÍTICAS SOCIALES

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CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Madrid, 2 de julio de 2014

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XI Encuentro de Cooperación Farma-Biotech

1. The Institution

IBiS is a comprehensive and multidisciplinary biomedical research facility focused on translational research on the most prevalent diseases

IBIS is supported by all the major regional and nation-wide research agencies in Andalusia and Spain

IBIS promotes the **transfer of knowledge** to the clinical setting

Hospital Universitario Virgen del Rocío is one of the largest university hospitals in Spain providing health coverage to over half million people and acts as a hospital of reference in some of its specialized areas.

XI Encuentro de Cooperación Farma-Biotech

1. The Institution

Dr. Rocío García-Carbonero.

- Consultant in Medical Oncology in Virgen del Rocio University Hospital (Seville, Spain) since 2007, Associate Professor at Universidad de Sevilla & Co-Principal Investigator of the Molecular Oncology & Developmental Therapeutics Laboratory at IBiS.
- Member of the executive board of the Spanish Society of Medical Oncology (SEOM), of the executive board of the European Society of Neuroendocrine Tumors (ENETS) and member of the Scientific Advisory Group for Oncology (SAG-O) of the EMA (2008-present).
- More than 70 peer-reviewed scientific papers, with over 2,500 citations and an H-Index of 22, focused on early drug development and validation of biomarkers in the field of GI oncology.

Dr. Amancio Carnero.

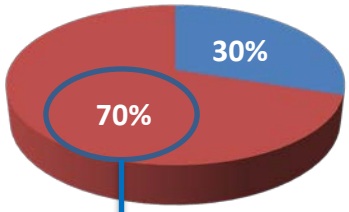
- Principal Investigator of the Laboratory of Molecular Biology of Cancer at IBiS since 2009, focused on the identification of new molecular targets involved in cancer development and progression, and in the identification of novel antitumor targets and compounds.
- Ph.D. in Molecular Biology at the Univesidad Autónoma de Madrid, Spain (1994) for his work on ras signal transduction pathway at the Instituto de Investigaciones Biomédicas (CSIC).
- Postdoctoral Fellowship granted by the European Molecular Biology Organization (EMBO), (1997-2000); Senior Research Fellow at the Institute of Child Health, London, UK.
- More than 80 publications in the field of identification and characterization of genes with therapeutic relevance in cancer and validation of new therapeutic targets.

2. The Product

a) Target Indications

- **Colorectal cancer (CRC)** is the third most common tumor worldwide and is responsible for 8% of cancer related deaths.
- Once the tumor has progressed beyond surgical resectability the disease is **essentially incurable**.
- Several combination chemotherapy regimens including fluoropyrimidines and oxaliplatin and/or irinotecan, with or without monoclonal antibodies targeting VEGF (bevacizumab) or EGFR (cetuximab, panitumumab), remain the mainstay of care in metastatic CRC (mCRC). **Response rates**, however, are observed **in only 40-60% of patients** and median survival does not generally exceed 24 months.

INITIAL DIAGNOSIS



Almost 50% of these patients will progress to advanced stages and develop metastases.

The overall 5-year survival is around 50%, decreasing to 10% in patients at stage IV

2. The Product

a) Target Indications

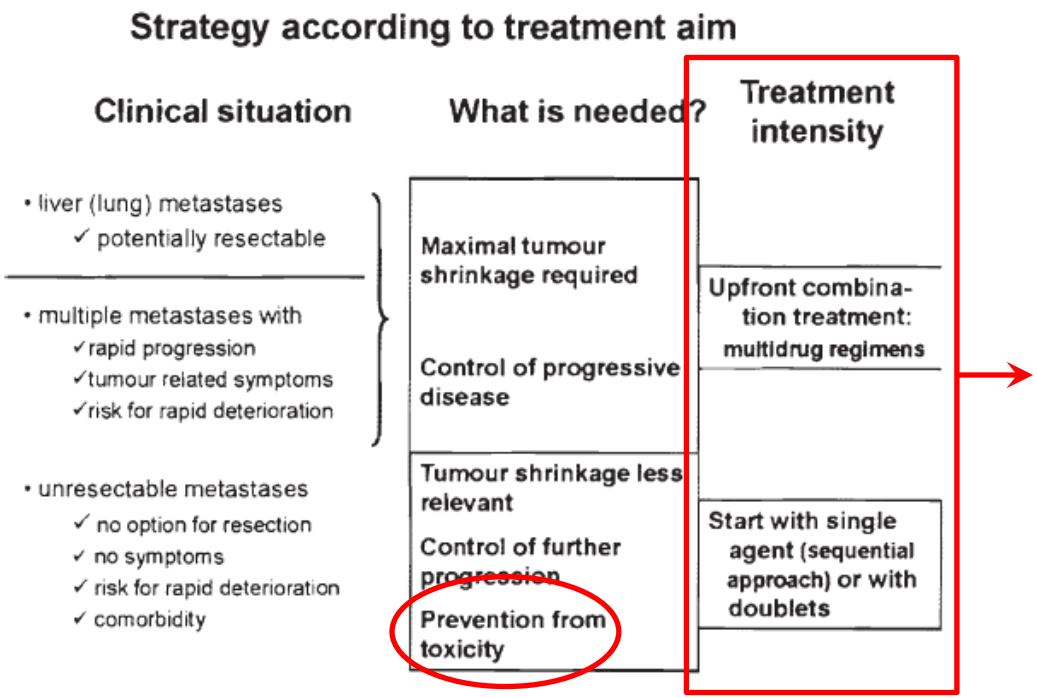


Figure 1. Strategy for the treatment of metastatic CRC (modified from Expert discussion ESMO/WCGIC Barcelona june 2009)

Source: Annals of Oncology 21 (Supplement 5): v93–v97, 2010

PERSONALIZED MEDICINE IN CRC

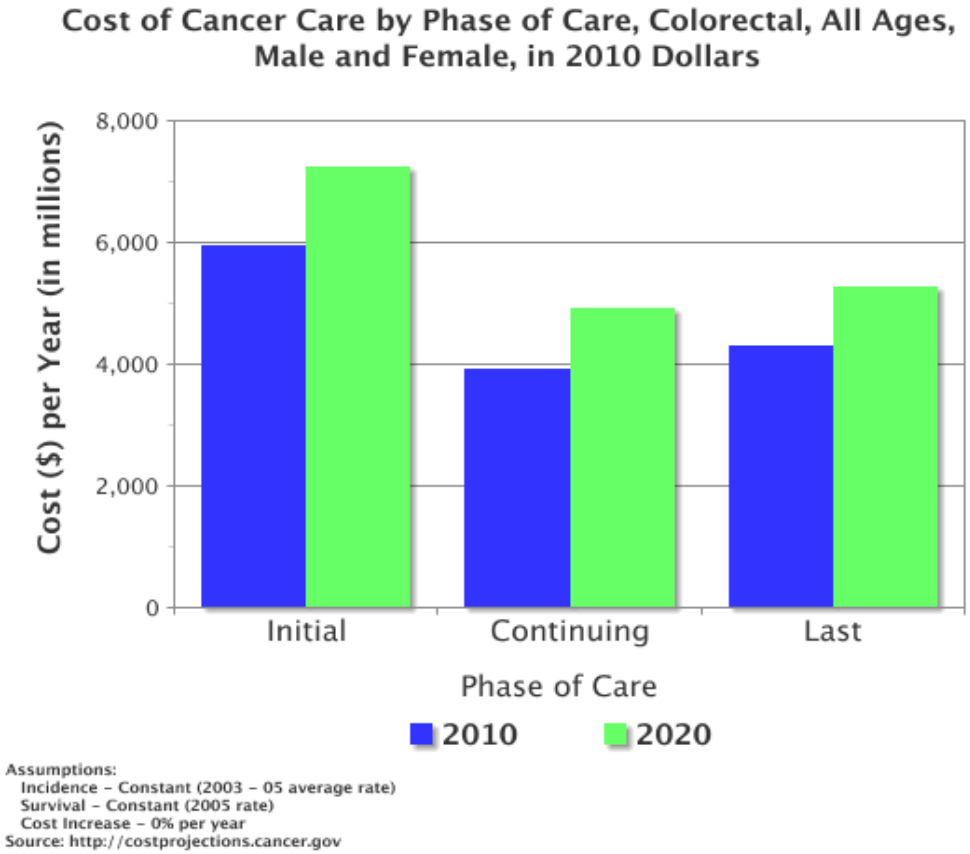
- **Prediction** of the response to treatments **needed.**
- **Benefits the patient by reducing drug toxicity and optimizing patient outcome.**
- **Reduces costs for an already burdened health system.**
- **Future in the management of CRC in combination with minimally invasive surgical techniques.**

2. The Product

a) Target Indications

- **Annual expenditure for CRC** was conservatively estimated at **$14 billion in 2010** by the National Institutes of Health (NIH) and that is estimated to increase for 2020.

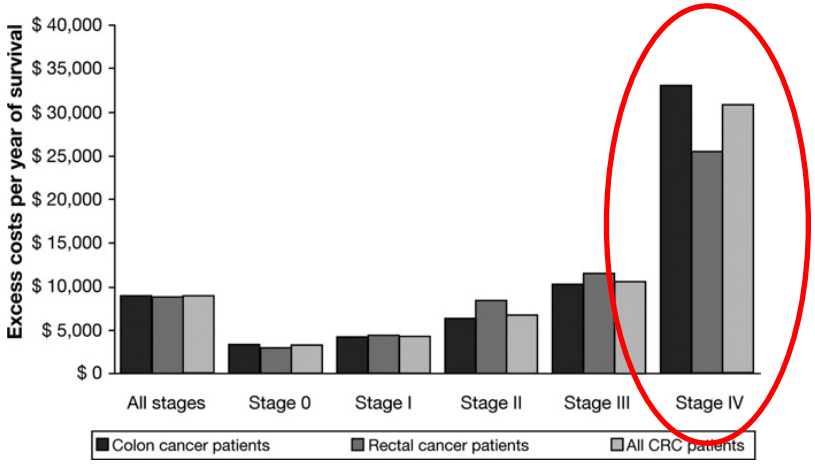
- In 2010, Medicare spent an estimated **$7.4 billion dollars on CRC treatment**, which is 85% of the National Cancer Institute's (NCI) estimate for expenditures for those 65 and over.



Source: US National Institutes of Health

2. The Product

a) Target Indications



Source: Clinical Gastroenterology and Hepatology 2009;7:198–204

Substantially **higher costs for later-stage cancers** may indicate that these cancers **receive more drastic or expensive treatments**.

Important to focus on earlier detection of CRC and **reduction of treatment costs as a way to reduce medical expenditures.**

Medicare Cost Savings (in $billions)	Set A: Lower Treatment Costs		Set B: Intermediate Treatment Costs		Set C: High Treatment Costs	
	FOBT	COL	FOBT	COL	FOBT	COL
CRC Prevention	$ 6.8	$ 7.4	$ 12.2	$ 13.3	$ 17.5	$ 19.1
Reduction in Treatment Costs	$ 1.0	$ 1.6	$ 1.2	$ 2.5	$ 1.3	$ 3.3
Total Medicare Treatment Cost Savings	**$7.8**	**$9.0**	**$13.4**	**$15.7**	**$18.9**	**$22.4**
Screening Cost (Screening and Surveillance)	$ (0.1)	$ (0.7)	$ (0.1)	$ (0.7)	$ (0.1)	$ (0.7)
Total Medicare Savings	**$7.7**	**$8.3**	**$13.3**	**$15.0**	**$18.8**	**$21.7**

FOBT- Fecal occult blood testing; COL- colonoscopy
Source: National Colorectal Cancer Roundtable. Sep 2007

2. The Product

a) Target Indications

- Biomarkers are also frequently used in late stage clinical trials due to its potential in prognosis, prediction, patient screening and stratification.

- Oncology is currently the most attractive field in clinical trials.

Table 3.20: Global biomarker late stage clinical trials applications market, by diseases 2007-2014f ($m)

Application	2007	2008	2009	2014f	CAGR% (2009-2014)
Oncology	93.1	113.8	135.5	405.7	24.5
Cardiovascular	78.4	95.8	117.2	314.4	21.8
Neurological	36.7	44.9	58.6	162.3	22.6
Autoimmune	24.5	29.9	36.6	91.3	20.1
Others	12.2	15	18.4	40.5	17.1
Total	**244.9**	**299.4**	**366.3**	**1014.2**	**22.6**

Source: author's analysis — Business Insights Ltd

Source: Business Insights: Biomarkers in Late Stage Clinical Trials: Applications, opportunities and activities of leading players. 2010

2. The Product

a) Target Indications

The present technology **provides methods and tools for predicting treatment outcome of patients with advanced colorectal cancer (CRC) treated with FP-based CT,** in particular tumor response, progression-free and overall survival

The **diversity of treatment options** for metastatic CRC (cytotoxic chemotherapy and monoclonal antibodies) makes necessary:

to have a **method to predict the response of patients to treatments**

and thereby **facilitate the choice of the most appropriate therapy in each case**

2. The Product

a) Target Indications

Our research group has developed a method to predict the efficacy of fluoropyrimidine-based chemotherapy in patients with advanced Colorectal Cancer

set of microRNAs

Expression profile **predictive** of the **clinical outcome of chemotherapy** (fluoropyrimidine and oxaliplatin or irinotecan): tumor **response, progression and/or overall survival**

mRNA microarrays

Expression profile **predictive** of the **clinical outcome of chemotherapy** (fluoropyrimidine and oxaliplatin or irinotecan): tumor **response, progression and/or overall survival**

Enable to select the most effective treatment for an individual patient

2. The Product

d) Current status of development

set of microRNAs

78 patients with advanced CRC treated with FP-based chemotherapy

- **Training Cohort** (667 miRNAs assessed by TLDA) — 39 patients
- **Validation cohort** (8 predictive miRNAs assessed by RT-qPCR) — 39 patients

mRNA microarrays

123 patients with advanced CRC treated with FP-based chemotherapy

- **Training cohort** (whole genome expression analyses) — 37 patients
- **Expanded validation cohort** (RT-qPCR analyses) — 86 patients + 33 controls

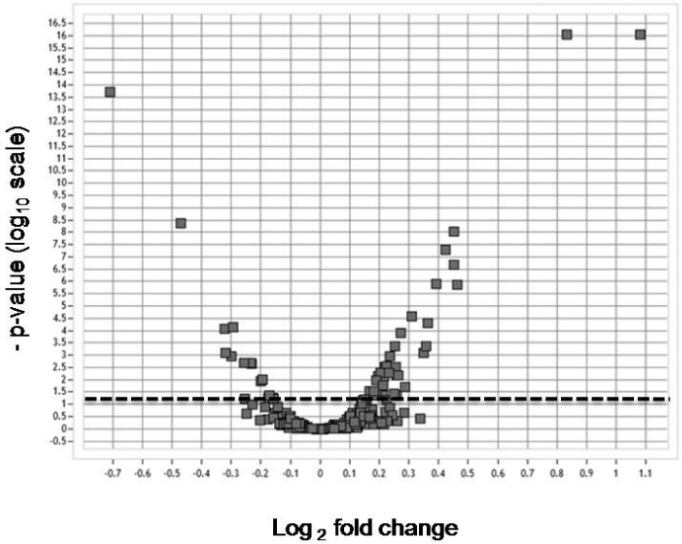
2. The Product

b) Innovative mechanisms of action

miRNA Signature

MicroRNAs	R vs NR (-ΔΔCt)	Adjusted p-values*
miR O*	0.863	**0.042**
miR-A	0.706	**0.042**
miR-B	0.864	**0.006**
miR-C	0.952	**0.018**
miR-D	1.162	**< 0.001**
miR-E *	0.877	**0.006**
miR-F	-0.560	**0.042**
miR-G *	0.715	**0.016**

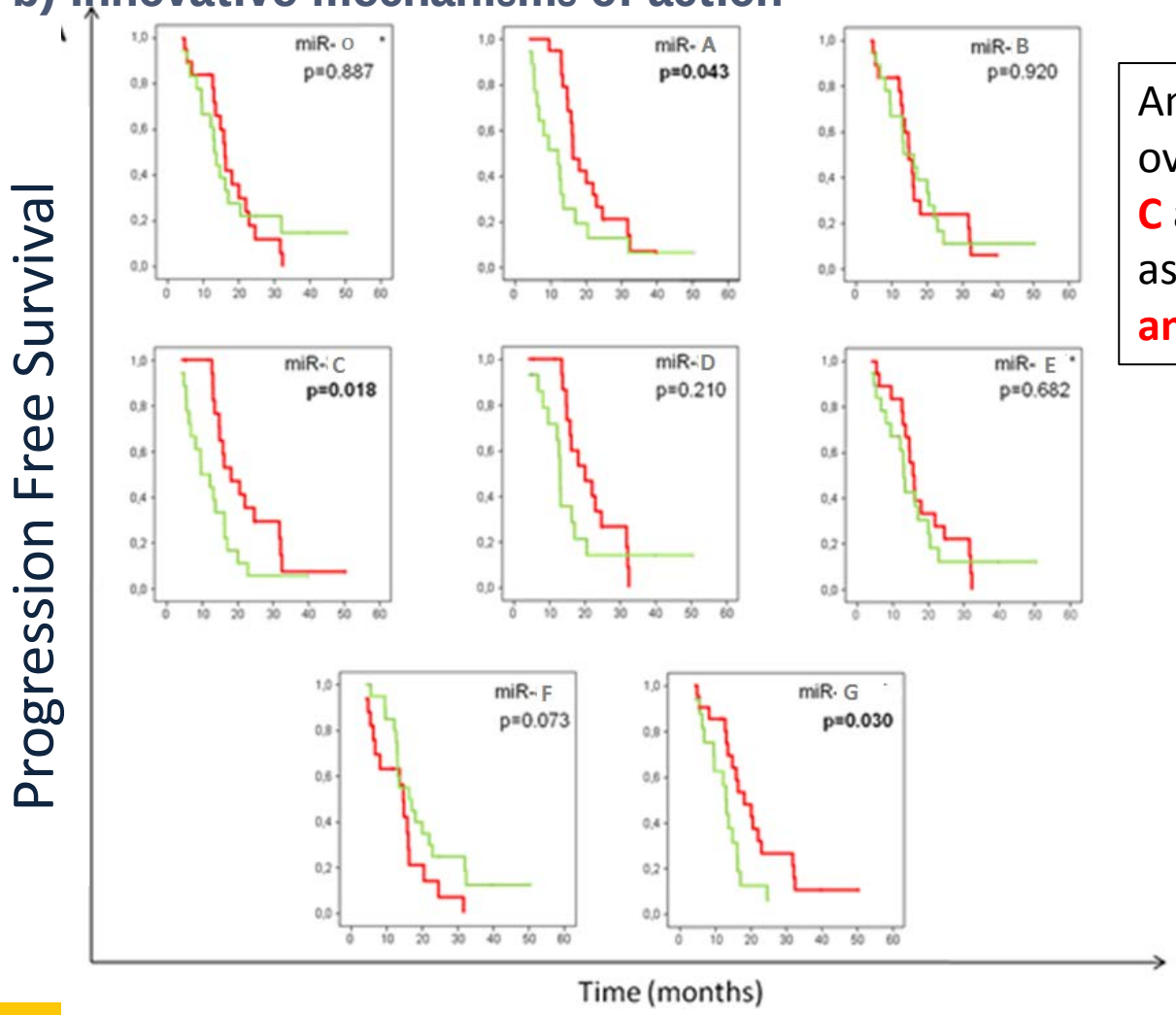
8 differentaily expressed miRNAs predictive of response to CT were detected at least in 50% of tested samples in each group



46 miRNAs were differentially expressed in patients with advanced CRC responding vs non-responding to FP-based chemotherapy through TLDA

2. The Product

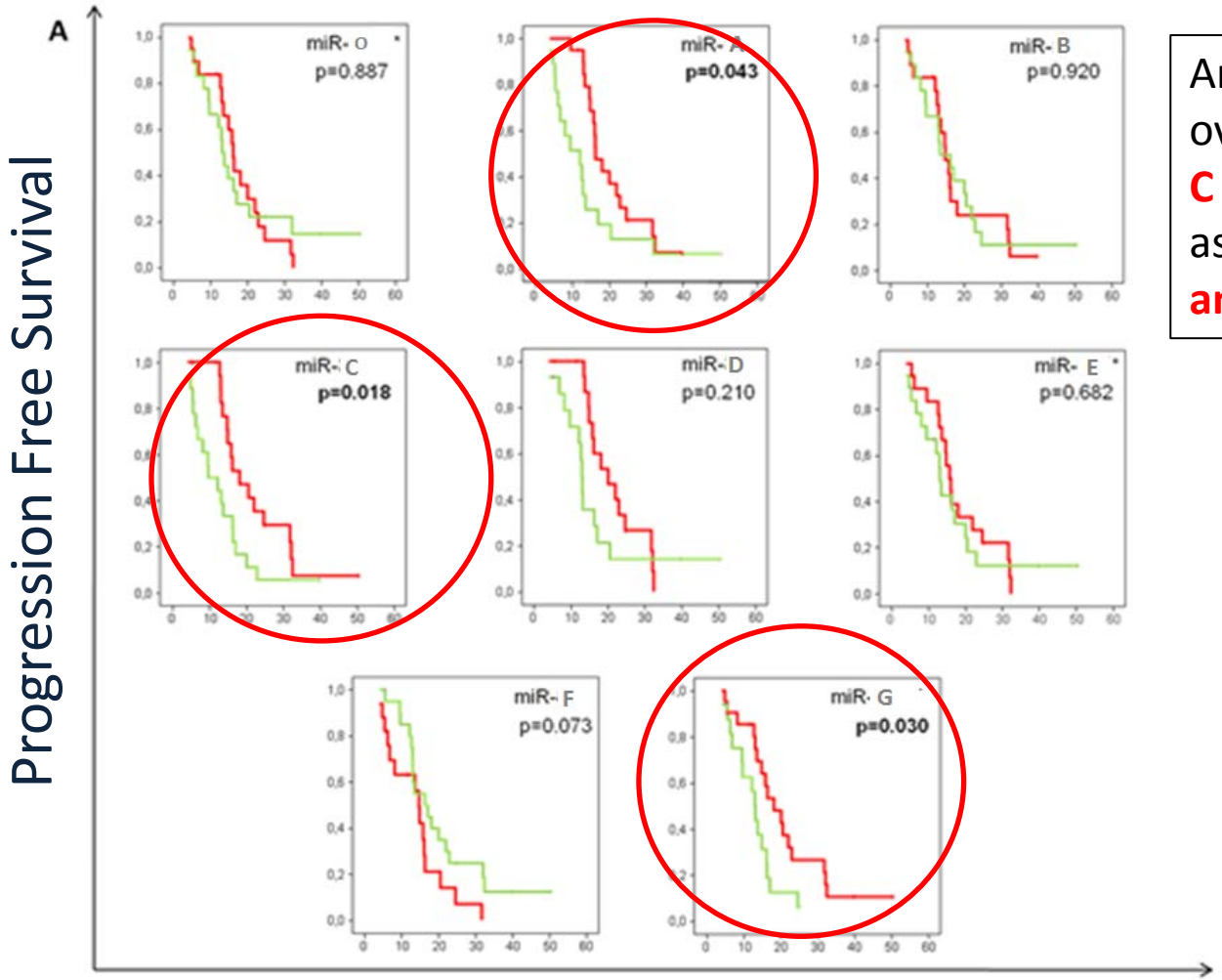
b) Innovative mechanisms of action



Among tested miRNAs, overexpression of **miR-A, miR-C** and **miR-G** was significantly associated with improved **PFS and OS (p<0.05)**

2. The Product

b) Innovative mechanisms of action



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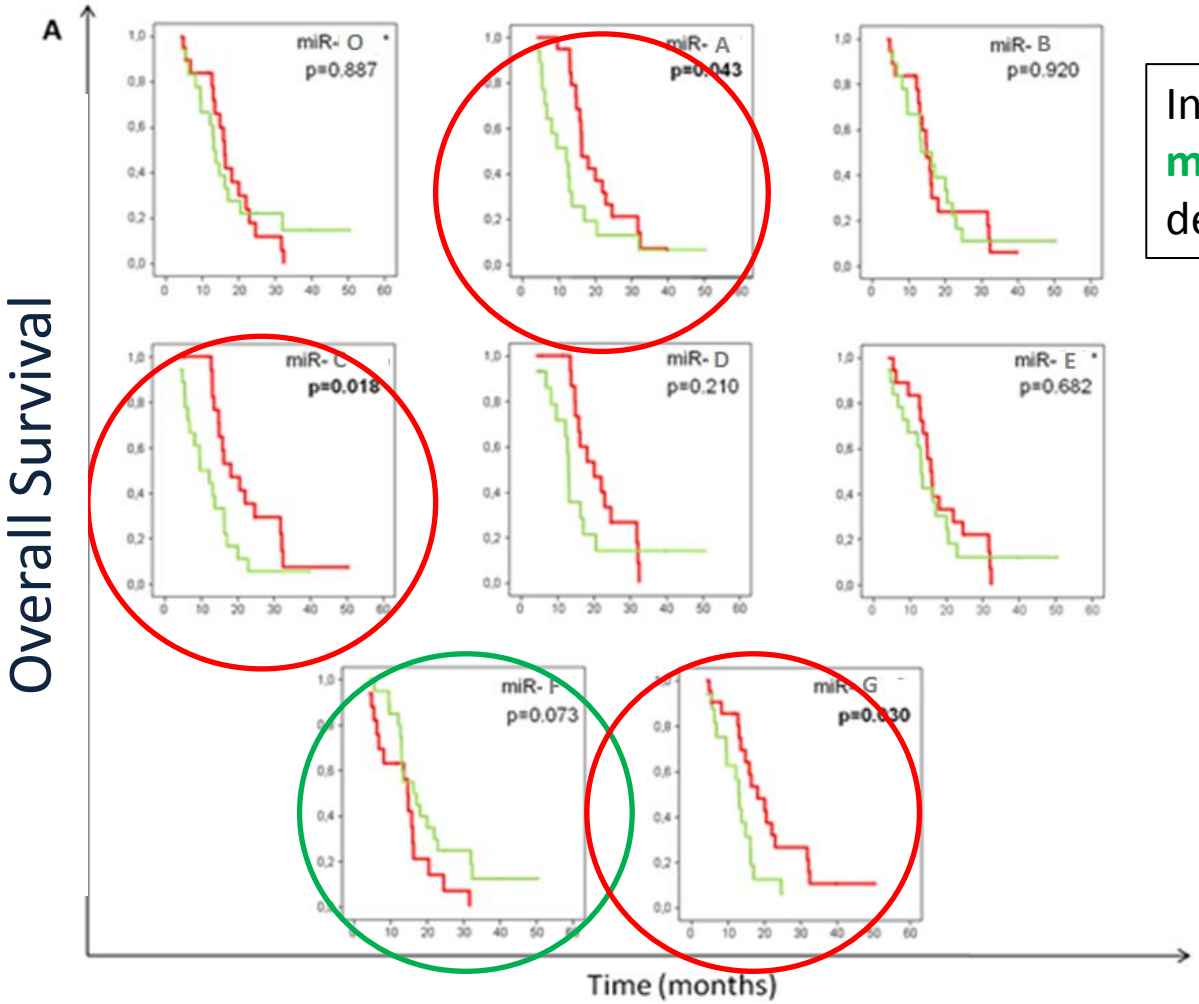
2. The Product

b) Innovative mechanisms of action



In addition, overexpression **of miR-F** was associated with decreased **OS (p<0.05).**

2. The Product

b) Innovative mechanisms of action

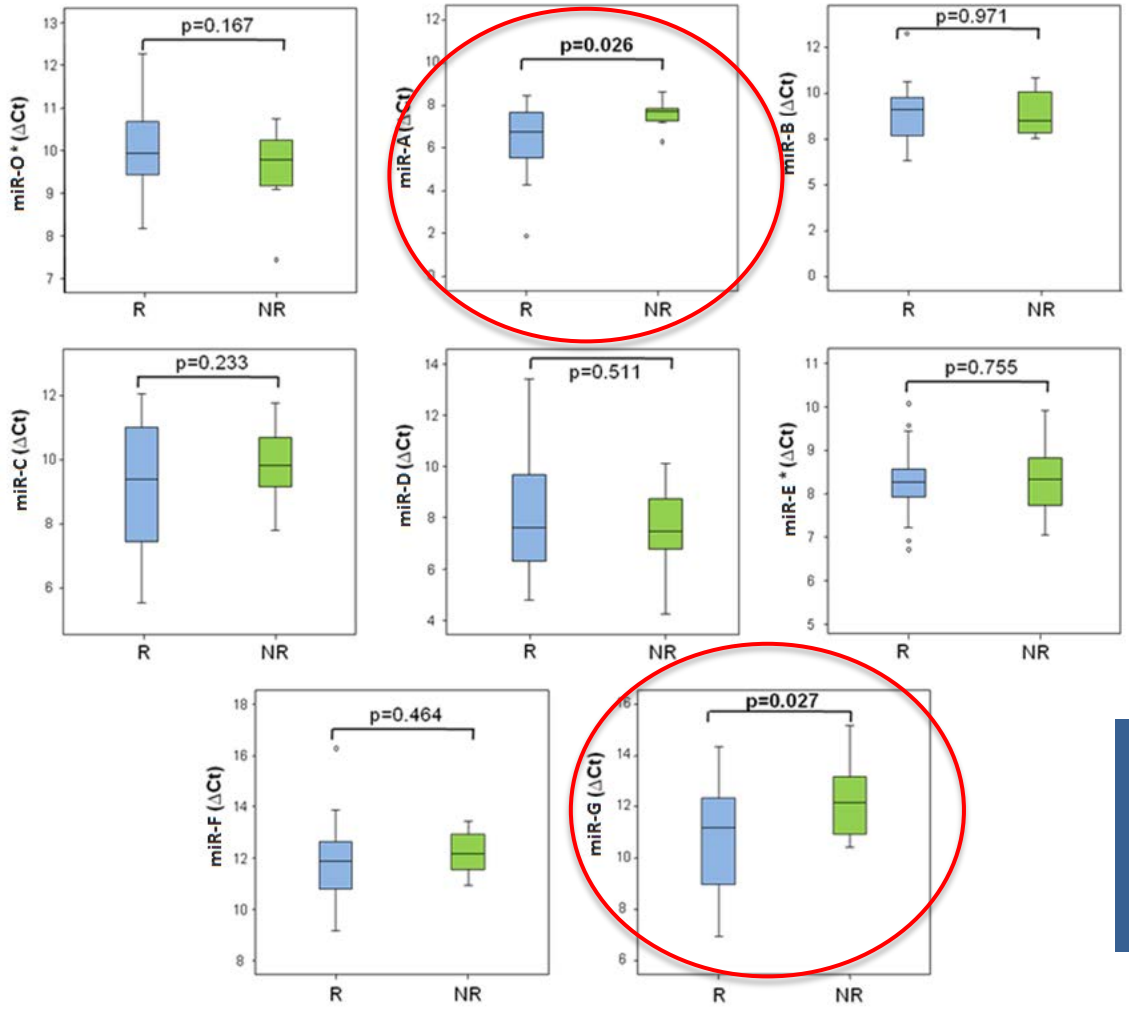
Multivariate Analysis confirmed these miRNAs as independent predictors of Outcome

VARIABLES	PFS				OS			
	Univariate Analysis		Multivariate Analysis		Univariate Analysis		Mutivariate Analysis	
	HR (95% CI)	*p-value*	HR (95% CI)	*p-value*	HR (95% CI)	*p-value*	HR (95% CI)	*p-value*
Age	0.99 [0.95-1.02]	0.357	0.99 [0.95-1.04]	0.765	1.01 [0.96-1.05]	0.746	1.06 [1.01-1.12]	**0.027**
Sex	0.51 [0.24-1.07]	0.069	0.60 [0.26-1.40]	0.232	0.42 [0.16-1.10]	0.069	0.17 [0.05-0.54]	**0.003**
miR- A	2.12 [1.05-4.29]	0.043	2.52 [1.18-5.42]	**0.017**	2.65 [1.06-6.67]	0.035	2.61 [0.86-7.92]	0.091
miR- C	2.27 [1.12-4.58]	0.018	3.02 [1.34-6.83]	**0.008**	2.53 [0.95-6.80]	0.018	1.40 [0.42-4.69]	0.584
miR- G	2.34 [1.11-4.93]	0.030	2.50 [1.00-6.05]	**0.050**	3.46 [1.26-9.53]	0.008	1.99 [0.62-6.36]	0.243
miR- F	0.45 [0.22-0.94]	0.073	0.40 [0.16-0.90]	**0.027**	0.26 [0.10-0.71]	0.017	0.15 [0.04-0.47]	**0.001**

Stable disease (SD); partial response (PR); complete response (CR); progressive disease (PD).

2. The Product

b) Innovative mechanisms of action



Independent Validation

Overexpression of **miR-A** and **miR-G** was significantly associated with response to chemotherapy

These miRNAs are suitable predictors of response.

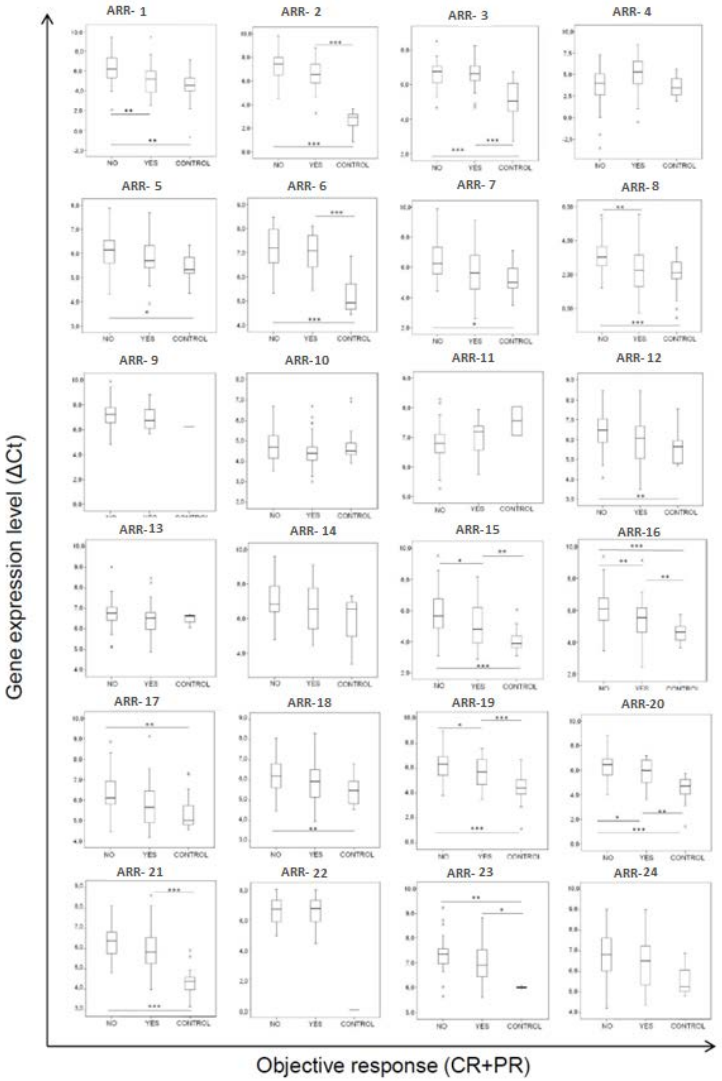
2. The Product

b) Innovative mechanisms of action

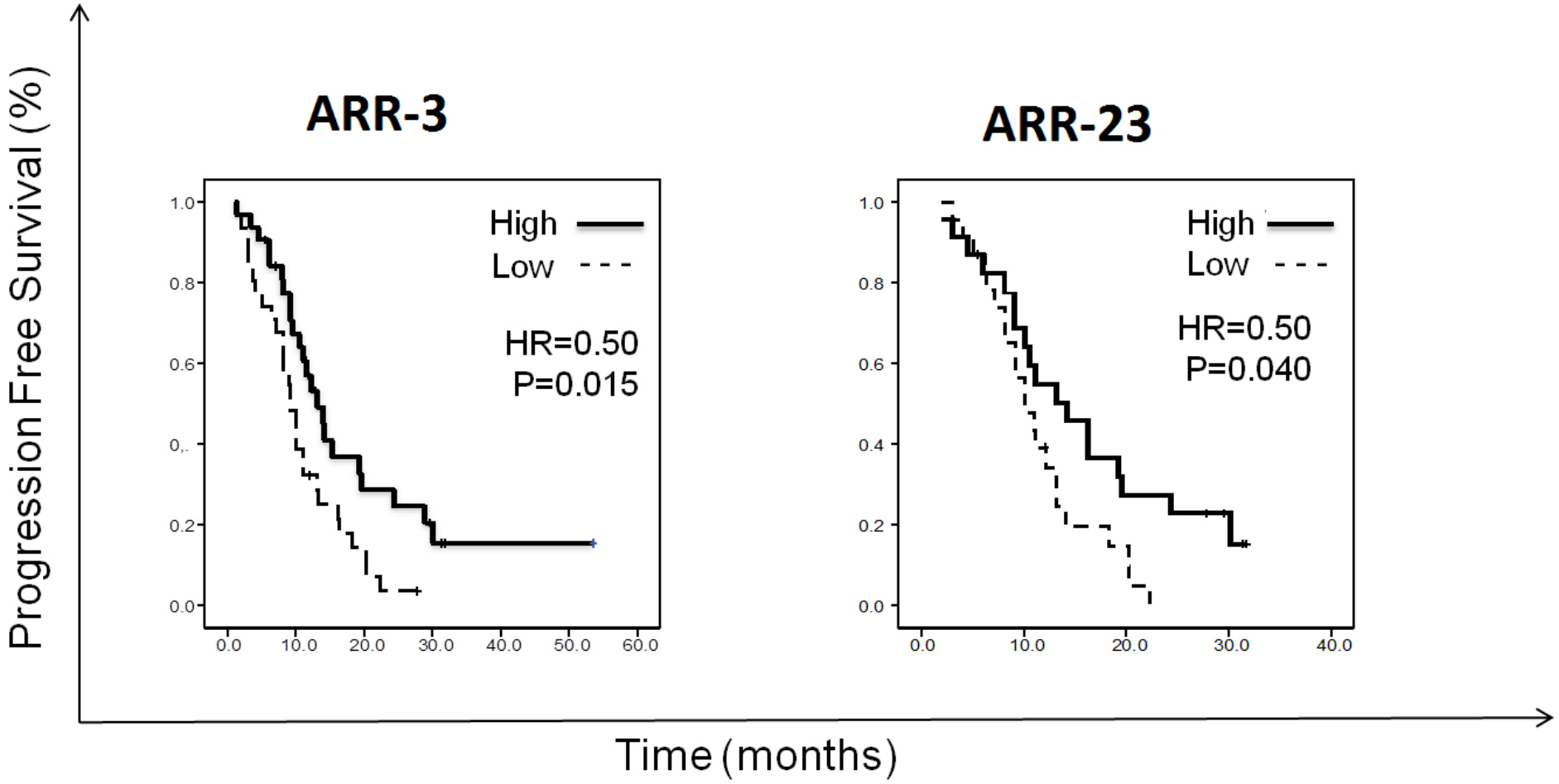
mRNA expression profiling (Training Set)
Whole genome expression microarrays
Affimetrix HG-U133 Plus 2.0

Validation Cohort
A **set of 161 genes that were differentially expressed** between Responders vs Non-Responders to chemotherapy were selected for further assessment by RT-qPCR
(TLDA 77900 HT microfluidic cards)

24 genes were validated as significant predictors of response to chemotherapy



2. The Product



2. The Product

c) Differential features facing the market

What`s new in colorectal cancer research and treatment?
Research is always going on in the area of colorectal cancer. Scientists are looking for ***causes and ways to prevent colorectal cancer*** *as well* ***as ways to improve treatments****.*

Genetics
Tests (including Oncotype Dx® Colon Cancer Assay, ColoPrint®, and ColDx™) have been developed that look at the activity of many different genes in colon cancer tumors. These tests can be used to help predict which patients have a higher risk that that the cancer will spread. ***So far, though, none of them have been shown to help predict who could benefit from chemo or other treatments****.*

2. The Product

c) Differential features facing the market

Tool/ Biomarker	Status
Prediction of Response to Primary Treatment for CRC	
Pathologic & Molecular Staging	• Although it has achieved standard of practice for breast cancer and significant use in selected cases of malignant melanoma, the sentinel lymph node biopsy has not, to date, been widely applied to CRC surgery. • A variety of molecular assays have been developed to assess the status of CRC surgical resection margins, but this approach has been limited to research studies.
Oncotype Dx Colon Cancer Test	• The current format of this test is not designed to predict the response of a specific therapy.
ColoPrint	• Prognostic gene signature but not predictive.
Circulating Tumor Cells	• To date, the ability of CTC determinations to guide therapy selection and the changing of therapy in CRC has not been confirmed in prospective clinical trials.
Cytotoxic Chemotherapy for CRC: Biomarkers for the Prediction of Efficacy and Toxicity	
5-Fluorouracil & Capecitabine (TYMS, DPYD, MTHFR, DNA Mismatch Repair Status)	• High percentage of false-negative results, possibly due to incomplete sequencing and pathway analyses. • Prospective, randomized studies of biomarker-driven selection or rejection of irinotecan for the treatment of recurrent or metastatic CRC are lacking. • Tests are not currently recommended for daily clinical use by regulatory authorities or specialty societies. • For an individual patient with high-risk primary CRC, there may be germline and tumoral genomic information whose eventual clinical validation can lead to a specific selection of agents, rather than using the "one-size-fits-all" approach.
Irinotecan (Topoisomerase I, UGT1A1 & other)	
Oxaliplatin (ERCC1, ERCC2, RRM1, GSTP1, POLB and other)	

Source: American Journal of Clinical Pathology, 2010, 134, 478-490.

2. The Product

c) Differential features facing the market

Tool/ Biomarker	Status
Therapeutic Antibodies for CRC: Biomarkers for the Prediction of Efficacy and Toxicity	
Anti–EGFR Antibody Drugs (Cetuximab and Panitumumab)	
EGFR Mutation	• EGFR expression at the protein or mRNA levels has not correlated with drug response. • EGFR somatic gene mutation (major predictor of efficacy of anti-EGFR small molecule drugs in non–small cell lung cancer), has not successfully predicted response to anti-EGFR antibodies.
KRAS Mutation	• Actual mutation test has not been standardized, and fully validated tests appear to be lacking in clinical practice • Currently, there is no US FDA clearance or approval for a KRAS mutation test. • Concern about whether frameshift and missense mutations should also be detected and reported and whether codon 61 should be sequenced to search for mutations in addition to codons 12 and 13
BRAF Mutation	• BRAF inhibitors are not currently under evaluation for the treatment of CRC
Other (PIK3CA Mutation, p53 Mutation, PTEN Expression, Fcγ Receptor Status & ADCC)	• Data are limited for these biomarkers, and widespread adoption of their testing in CRC is not anticipated for the foreseeable future.
Bevacizumab	
	• To date, no biomarkers have emerged that are capable of predicting efficacy for this agent

Source: American Journal of Clinical Pathology, 2010, 134, 478-490.

2. The Product

c) Differential features facing the market

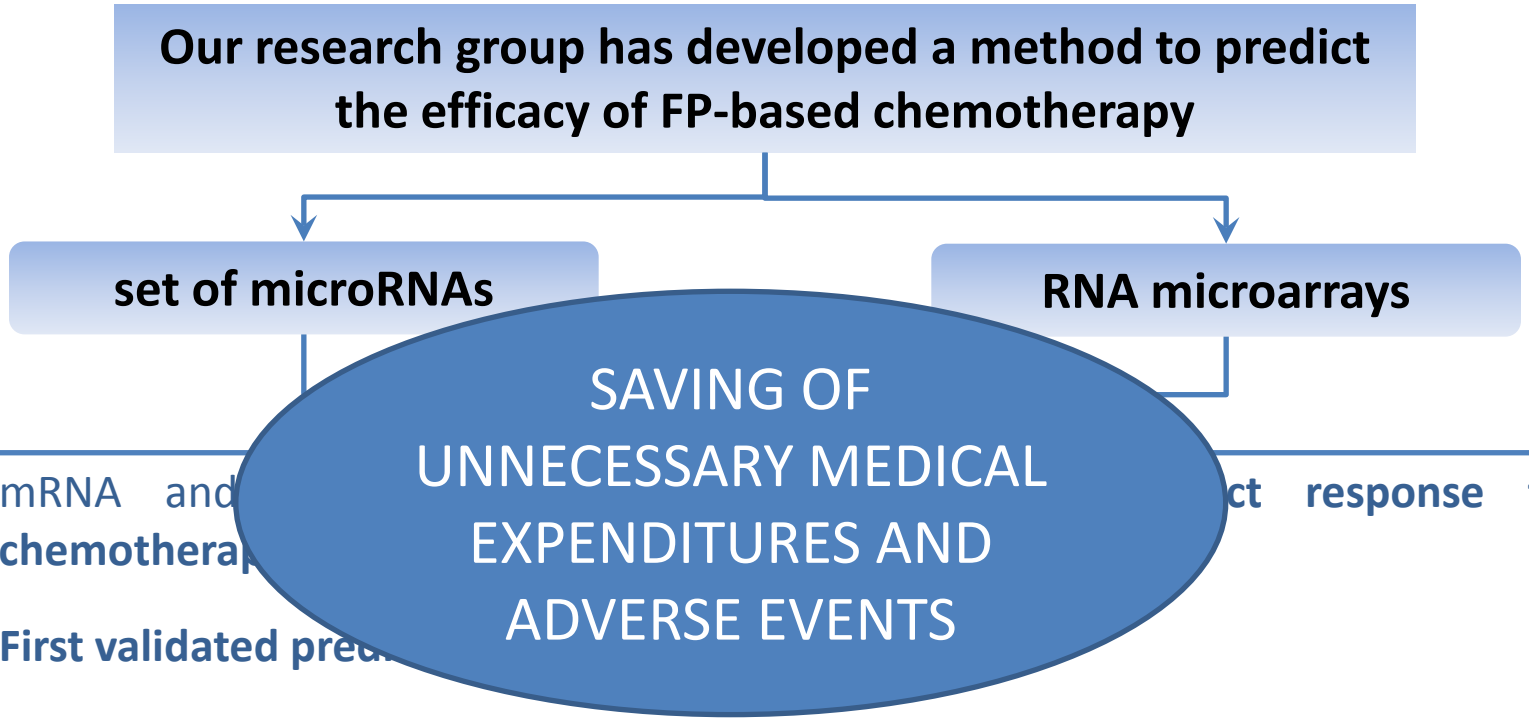
- With the exception of **RAS mutations** as predictors of resistance to EGFR-targeted therapy, **no validated biomarker to date has been able to assist clinicians in the selection of the most appropriate treatment regimen** for a specific patient.

- Many attempts have been made over the past decades to identify molecular markers predictive of response to chemotherapy in the context of CRC. However, none of these putative markers have been implemented in clinical practice due to their **poor prediction accuracy** and also to the **lack of reproducibility** across different studies and patient populations.

- While gene expression profiling has been widely applied to CRC for diagnosis, classification and prognosis, studies evaluating its potential role to predict response to medical therapy are still scarce. Indeed, most available data to date derive from preclinical studies.

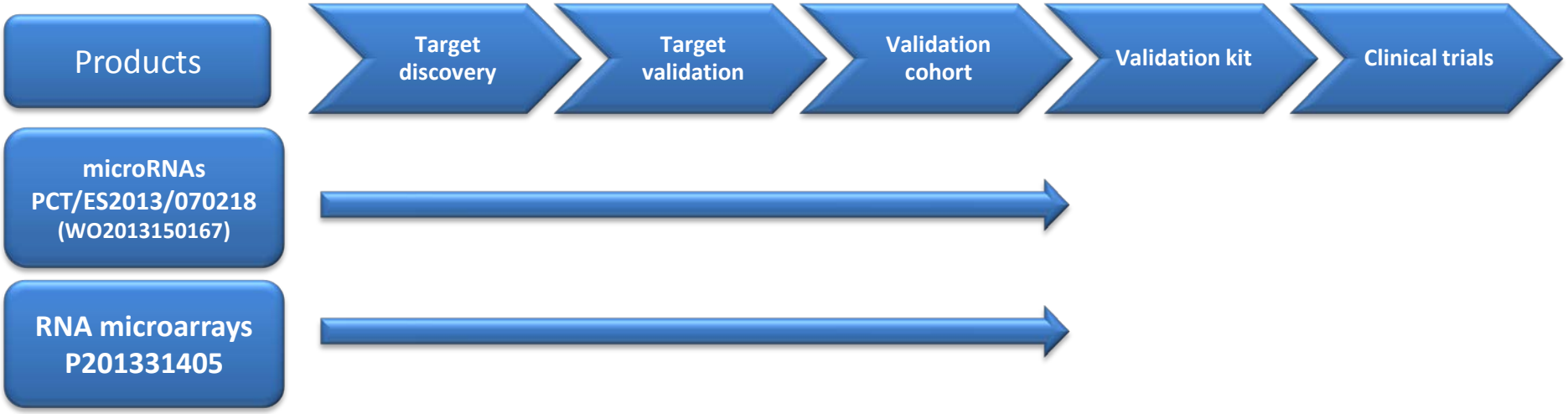
2. The Product

c) Differential features facing the market



2. The Product

d) Current status of development



- **Next steps should be validated in clinical trials**

2. The Product

e) IPR protection

microRNAs

PCT/ES2013/070218
(WO2013150167)

Filed in Apr 2013
(priority date: Apr 2012

- A **method of predicting response** of a human subject, suffering from advanced CRC, to chemotherapy using, as an indicator, **expression levels of one or more of the identified miRNAs.**
- A **kit** comprising at least one oligonucleotide(s) capable of hybridizing with any one, two or more, and preferably all, of the identified miRNAs.

Entry to national phases expected by Oct 2014

RNA microarrays

P201331405

Filed in Sep 2013

- A **method for obtaining useful data for predicting response** of a human subject, suffering from **advanced CRC, to chemotherapy**.
- A **kit or medical device** which comprises the needed molecular tools for quantifying the expression levels of the identifies genes.
- A **microarray** which comprises oligonucleotides designed from a known sequence or the genes mRNA.

International Patent Application to be filed by Sep 2014

2. The Product

f) Pitfalls & Risks to be considered

- **Larger and prospective confirmatory studies are required** to successfully optimize treatment selection in each individual patient in routine clinical practice. Clinical trials to be conducted in a controlled, prospective manner before they can become universally accepted.

- **Validation in more widely available and/or less invasive samples >** such as formalin-fixed paraffin-embeded tumor samples or peripheral blood miRNA or CTCs.

- **Development of a mathematical model or algorithm** as a practical tool for modeling and exploiting the results obtained in the analysis needed.

3. Partnering opportunities

- Looking for a partner interested in a **license and/or a collaboration agreement (First Option Agreement)** to further develop and exploit this innovative technology.

- Open to establishing partnerships for **co-development** of the technology before reaching the market and highly interested in applying to different funding calls, mainly to **Horizon 2020.**

NEXTS STEPS OF DEVELOPMENT

- Further validate the present biomarkers in a larger and prospective clinical confirmatory study.

- Develop a kit comprising the needed molecular tools for quantifying the expression levels of the identified RNAs.

THANK YOU

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